

REMARKS

Claims 18-27 are pending. Claims 1-17 are cancelled without prejudice and new claims 20-27 are added, wherein new claims 21-27 are essentially duplicates of previously pending claims, amended to be ultimately dependent on claim 18. As such, none of these amendments constitutes new matter. Cancellation of subject matter herein is without prejudice to the prosecution of said subject matter in other patent applications.

The previously pending claims are rejected as unenabled, lacking description, anticipated and/or obvious. For reasons to be set forth below, the rejections should be withdrawn and the presently pending claims should be allowed to issue.

1. The Claims Are Definite

Claim 18 is rejected under 35 U.S.C. §112, second paragraph, as indefinite. The Examiner states that step c) does not state which “parental maize line” is the recurrent parent.

As suggested by the Examiner, claim 18 is amended to insert the phrase “of interest”, thereby obviating the rejection, which should be withdrawn.

2. The Claims Are Enabled

Claims 1-3, 5-6, 8, 10, 12, 14, 15, 18 and 19 are rejected, under 35 U.S.C. §112, first paragraph, as allegedly failing to comply with the enablement requirement because the claims allegedly “contain subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.”

Applicants respectfully disagree with the rejection, and submit that the pending claims, as amended, are fully enabled by the originally filed specification, drawings and claims.

According to the Examiner, the presently claimed invention is not enabled because it broadly claims applicability to different maize lines and plant types while only disclosing the use of the A188 and Hi-II lines.

Applicants note that use of maize genotypes other than those specified in the present specification merely involves routine crossing of a first “elite” maize line, unsuited for transformation, to a second, more easily transformable, cell line, to generate a hybrid. At the time the present invention was filed, the skilled artisan would have been aware of various elite and transformable cell lines. The method of generating hybrids from such lines is described in the specification at page 28, line 25 to page 31, line 3.

The Examiner has indicated that lists of suitable elite and transformable maize lines should be expressly provided in the specification. Applicants respectfully disagree. Such lists would, at the time the instant invention was filed, have been available to the skilled artisan. Because “a patent need not teach, and preferably omits, what is well known in the art,” (MPEP 2164.01, citing *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984)), the instant invention is therefore fully enabled for applicability to different maize lines. Applicants therefore respectfully request the Examiner to withdraw the rejection.

Since the claims directed to utilization of non-maize plant species are canceled (claims 6 and 15) the Examiner is requested to withdraw the lack of enablement rejection based on alleged lack of evidence for use of plants other than maize.

The Examiner alleges that Applicants' arguments relating to the methods for analyzing and comparing genomes as being known in the art is not persuasive. The Examiner has cited prior grounds of rejection from page 8 of the Official Action mailed June 25, 2004. The Examiner reiterates that cited references Welsh *et al.* (Nucleic Acids Res. (1990) 18(24): 7213-7218; hereinafter "Welsh *et al.*") and Staub *et al.* (HortScience (1996) 31(5):729-741; hereinafter "Staub *et al.*") demonstrate the alleged unpredictability inherent in applying any marker system to a wide variety of genotypes. Applicants' reading of the cited references is contrary to the Examiner's interpretation. Applicants respectfully note that Welsh *et al.* describe an arbitrarily primed polymerase chain reaction (AP-PCR) method which enables the simple and reproducible fingerprints of complex genomes without requirement of prior sequence information. Welsh *et al.*, state at page 7216, column 2, paragraph 5, that "AP-PCR will work with most genome and species" (having tested it on rice, maize and human genomes). Thus the Examiner has not explained why Welsh *et al.*, does not in fact actually exemplify the level of skill in the relevant art and, indeed, enable the discrimination of related genomes. In addition, the closest prior art cited by the Examiner Ragot *et al.* (Techniques et utilisations des marqueurs moléculaires; Montpellier (France) 29-31 Mars 1994; Ed. INRA, Paris 1995 (Les Colloques, n72); pages 45-56; hereinafter "Ragot *et al.*") utilizes a RFLP based analysis in maize, demonstrating feasibility of the approach disclosed in the present Application.

Thus contrary to the Examiner's interpretation, the disclosed markers and method of analysis in the present Application is fully enabled, and so the rejection should be withdrawn.

Finally, the Examiner has rejected the Applicant's arguments with regard to the number of backcrosses including the arguments provided in the Perez declaration. The Examiner, citing a prior rejection (page 7, Official Action mailed June 25, 2004) concludes that transgene insertion into a genomic region of a non-agronomic, transformable parent is "more likely" than in the untransformable elite line DNA. The Examiner effectively excludes any reasonable possibility of vector integration into the genome derived from the agronomically important parent of the hybrid. Applicants respectfully disagree.

First, it is generally held that crop lines vary in their ability to handle the stresses of *in vitro* tissue culture, and the amenability to *in vitro* growth may present as much if not more of a barrier to transformation than inaccessibility of an individual genome to accept foreign DNA. By creating a hybrid of an easily transformable line with an elite line, the ability to handle the stresses of *in vitro* growth of the elite line is sought to be addressed. Once the *in vitro* growth barrier is overcome, all parts of a target genome exposed to vector DNA is equally accessible for integration irrespective of which parent it comes from. It is incorrect for the Examiner to reach a broad conclusion that even in a hybrid only the non-elite genome is accessible to integration by a vector DNA. Furthermore, as Ragot uses, as a parent strain, a transgenic elite line, clearly the insertion of a transgene into the elite chromosome is not impossible.

The Examiner has summarized the lack of enablement rejection based on backcrossing by stating that "... it is impossible to obtain truly isogenic lines if the transgene were originally inserted into the non-agronomic parent." Based on this statement, Applicants respectfully conclude that the Examiner has not properly interpreted the invention, a central feature of which is the insertion of the transgene into the *agronomic* parent. When the transgene is inserted into the agronomic parent genome, the formation of a truly isogenic line is indeed

achievable, by removing, through backcrossing, the non-agronomic DNA. Applicants therefore assert that the present claims requiring selection of a transformant with vector insertion in the agronomically important genome is fully enabled and overcomes the problem stated by the Examiner. For the reasons cited above, Applicants respectfully request that the rejection be removed.

3. **The Claims Are Supported By The Specification**

Claims 1-3, 5-6, 8, 10, 12, 14-15 and 18-19 are rejected under 35 U.S.C. §112, first paragraph for allegedly failing to comply with the written description requirement. The Examiner alleges that the claims contain subject matter which was not described in the specification to reasonably convey that the inventors had possession of the claimed invention at the time the application was filed.

The Examiner has contended that the claims lack written description support due to alleged absence of description of molecular markers and marker assay techniques. In fact, such description is fully provided in the specification at page 31, line 4 to page 39, line 24. The method of the present invention is divided into multiple interdependent steps each of which involves a set of probes or markers and assay techniques associated for markers and methods for use of the same. These steps include: a) general screening step for identifying transformants with correct integration of the vector; b) a step identifying the host genomic integration sites bordering the vector; and c) a method for identifying the parent strain of the genome into which the vector has integrated. Each of these sub-steps is fully compliant with the written description requirement under 35 U.S.C. §112, first paragraph, as discussed below, so that the rejection should be withdrawn.

As a first step to identify a suitable transformant, Example 3 specifies that DNA extracted from a hybrid transformant may be subjected to Southern blot analysis using a restriction enzyme cutting at least once in the T-DNA (page 31, line 27 to page 32, line 6). Applicants respectfully observe that any person skilled in the art will be easily able to perform such an analysis even without specific experimental details. The use of a restriction enzyme, the electrophoretic separation and transfer of DNA and probing of a membrane (i.e. Southern blot analysis) are routine steps very well known in the art. A practitioner utilizing a specific type of T-DNA vector will know what enzyme to use and may well have a certain preference from a choice of different enzymes. Thus the description for which exact enzyme to use or additional method steps is not lacking.

Similarly, the type of probe to be utilized in Southern analysis is described at page 32, line 16 to page 33, line 17. Suitable primers to generate a probe (SEQ ID NOS: 1-4) in a defined region of the vector, and rationale for choice of the region is also provided in the instant specification. A practitioner will be able to screen not only for transformants containing a T-DNA insert from the method steps described, but will also be able to determine if the integration event is a simple monocopy insertion or not. Applicants respectfully suggest that not only is this description compliant with the written description requirement but it also provides a skilled practitioner with broader guidance so as to adapt the strategy to alternative vectors and experimental set-ups.

Similarly, the second step of the analysis for identification of genomic sequences adjacent to vector insertion is fully described so that any skilled artisan will be easily able to practice the method. The procedure, based on genome walking is known in the art and is in fact available as a commercialized kit. The specification discusses the experimental strategy so that a

skilled practitioner will understand its basis and be able to practice it. Details relating to choice of restriction enzymes, primer pairs and hybridization conditions including duplex melting temperatures are provided in the specification for the T-DNA vector used in the instant Application. The Applicants note that the site of vector integration cannot be predicted in advance and will occur by random integration of a targeting vector into the genome of the hybrid recipient. Thus complete primer information and related details cannot be provided in advance for primers, markers or sequence information at the actual site of integration. A more generalized method of analysis using a large number of known genomic markers may be performed as an alternative to the present method. However as presently disclosed the specification contains a written description so that skilled practitioner may easily and fully practice the method of identifying an unknown genomic sequence adjoining the site of random vector integration, in full compliance with 35 U.S.C. §112, first paragraph.

Finally, the steps required to identify which parental genome of the hybrid contains the vector is fully described and compliant with 35 U.S.C. §112, first paragraph. The method described is dependent on the prior two steps described above. It utilizes the recovered borders of vector integration in step b), to identify which individual parent of the hybrid contains the vector. Highly specific integration related probes are used for restriction fragment length polymorphism analysis to identify the parent genome in which integration has occurred. Comparative analysis of hybridization patterns generated from individual parent DNA and hybrid transformant DNA to achieve this objective is disclosed in the filed specification. The actual procedure involves routine Southern blotting analysis in combination with the specific probe described above and will be specific for the site of integration in each transformant. As such the level of skill in the art and experimental strategy involved to practice the invention are

routine to the art related to the instant invention. In addition, the basic rationale and details of expected results (Figure 2) are provided in the specification as filed. The above demonstrates that the Applicants were in possession of a method to identify a vector positive transformant and further specifically identify which parental genome, in the hybrid was the site of vector integration.

In summary, Applicants contend that: (i) the steps of the methods are well enunciated in the claims and in the specification; (ii) multiple references, including references cited by the Examiner, at page 18 of the Response filed October 21, 2004, demonstrate that all the individual steps of the invention are well-known in the art including documents already of record demonstrating the existence of broad knowledge of these methods in the existing art at the time of filing of the invention. Therefore a person skilled in the relevant art would readily understand that the inventors had the combination of steps required to practice the claimed invention in their possession at the time of filing the application. For these reasons, Applicants respectfully request that the rejection be removed.

5. The Claims Are Not Anticipated

Claims 12 and 19 are rejected under 35 U.S.C. §102(b) as being anticipated by Ragot *et al.* According to the Examiner, the isotransgenic corn plant taught by Ragot *et al.*, only differs from the plant of the present invention by the method of making. Applicants respectfully disagree.

According to the Examiner, if vector integration has occurred in the non-agronomic portion of the hybrid genome, no amount of backcrossing will generate a truly isotransgenic plant. Ragot *et al.*, allegedly anticipating the present invention in fact use

backcrossing after vector integration into the non-desired genome (see page 46, 3rd and 4th paragraphs)! By the Examiner's own reasoning, the teaching of Ragot cannot achieve a truly isotransgenic line.

The Examiner further contends that there is no evidence of linkage drag having occurred in the plants of Ragot *et al.* and thus an isotransgenic line has been achieved by backcrossing. Applicants note that no evidence is presented in Ragot *et al.*, for example by providing sufficiently detailed sequence data at the boundaries of integration to formally demonstrate that their "isotransgenic" plant lacks any exogenous sequences derived from the genome in which the T-DNA originally inserted. In their concluding statement at page 55, Ragot *et al.*, state that "These results clearly demonstrate . . . for the production of near-isogenic lines through backcrossing."

Thus, by generally acceptable standards, but also by the Examiner's own admission, Ragot *et al.*, at best describes a very nearly isotransgenic plant. Since it may be safely concluded that the method of Ragot *et al.*, does not achieve true isogenic lines due to the theoretical impossibility of achieving this status even with an infinite number of backcrosses, a view also held by the Examiner, the present claims for a truly isotransgenic line are clearly distinct from the lines disclosed by Ragot *et al.*. Accordingly, it is requested that the rejection for lack of novelty be withdrawn.

6. The Claims Are Not Obvious

Claims 1-3, 5-8, 10, 12, 14-15, and 18-19 are rejected under 35 U.S.C. §103(a) as allegedly being obvious over Ishida *et al.*, (Nature Biotech. (1996) 14:745-750; hereinafter "Ishida *et al.*") in view of Does *et al.*, (Plant Mol. Biol. (1991) 17:151-153; "hereinafter "Does *et*

al.”), Hiei *et al.*, (Plant Journal (1994) 6(2):271-282; hereinafter “Hiei *et al.*”), Armstrong *et al.*, (Theoretical and Applied Genetics (1992) 84:755-762; hereinafter “Armstrong *et al.*”) and Ragot *et al.*. Applicants respectfully disagree.

The Examiner has used the following basis in alleging that when combined, the cited references render the present invention obvious. According to the Examiner, Ishida *et al.* teaches transformation of a hybrid plant, but does not teach selection of hybrid transgene positive primary transformants or backcrossing to produce an isotransgenic line; Does *et al.*, and Hiei *et al.*, teach methods of screening to identify transgene positive transformants using primers from flanking T-DNA sequences; Armstrong *et al.*, teach a method of RFLP analysis and a backcrossing strategy; and Ragot *et al.*, teach a method to rapidly obtain a (nearly) isotransgenic line by a backcrossing strategy used in conjunction with combined selection with an agent and molecular markers.

To establish *prima facie* obviousness, all the claim limitations must be taught or suggested by the prior art (*In re Royka*, 490 F.2d 981, 180 U.S.P.Q. 580 (C.C.P.A. 1974). *In re Wilson*, 424 F.2d 1382, 1385, 165 U.S.P.Q. 494,496 (C.C.P.A. 1970) states that “All words in a claim must be considered in judging the patentability of that claim against the prior art.” Applicant’s note that none of the above cited references considered singly or in combination suggest a specific step for the positive identification of a transgenic plant that contains T-DNA integrated only into the genome of the line of interest. Applicant’s reiterate that the selection step specified in claim 18 (b) clearly distinguishes the present invention from the combination of references cited by the Examiner.

It is interesting to note that even Ragot *et al.*, which teaches a high level of selective pressure to identify a suitable transgenic plant at each round of selection, does not

contemplate selection of a primary transformant with integration of T-DNA in the genome of the line of interest, as claimed in the present invention. Ragot *et al.*, in fact, fails to address or suggest any solution to the primary problem addressed by the instant invention, namely the inefficient transformability of agronomic, elite lines. Moreover, Ishida *et al.* omits a key element of the inventive method, namely, the elimination of non-agronomic genomic material. As the remaining references, Does *et al.*, Hiei *et al.*, and Armstrong *et al.* basically are technical articles supplying laboratory methods used in the art, and add nothing to complement the deficiencies of Ragot *et al.* and Ishida *et al.*, the combination of references cannot be considered to render the claims obvious. Furthermore, the combination of these references could only have been made with the benefit of hindsight, which is improper.

In addition, Applicants assert that the combination of references cited by the Examiner if anything, teaches away from practicing the present invention. Ragot *et al.*, teaches that obtaining an isotransgenic plant merely requires the application of adequate selection pressure at each stage of the backcross so that the undesired genetic elements are crossed out. At no point is the reverse strategy of screening for an integration event in the desired agronomically important genome suggested. When combined with the backcrossing and screening procedures described in the other references cited by the Examiner, one still does not arrive at the critical and distinguishing method step to obtain truly isotransgenic plants specified in the present invention. In essence, the combined teaching of all the cited references merely suggest that that aggressive and sensitive screening methods combined with an adequate number of backcrosses might achieve an isotransgenic plant with acceptable efficiency and so no further improvement may be required. Since none of the references alone or in combination suggest either the option

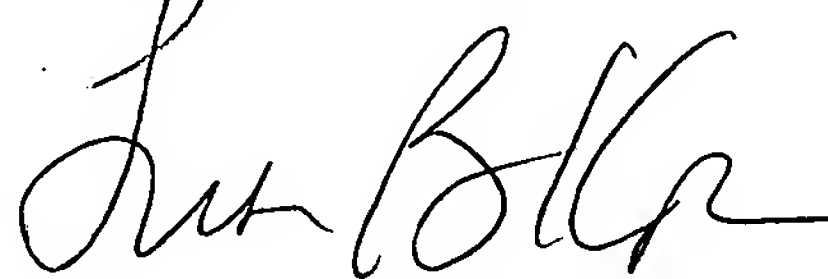
or feasibility of starting the backcrosses only after identifying a transformant wherein the genome of the line of interest has the T-DNA insertion, the present invention is not obvious. As such, Applicants respectfully request that the rejections under 35 U.S.C. §103(a) be withdrawn.

CONCLUSION

Applicants believe that in light of the foregoing amendments and remarks, the claims are in condition for allowance, and accordingly, respectfully request withdrawal of the outstanding objections and rejections. The Examiner is kindly invited to contact the undersigned if helpful to advance the application to allowance, which is earnestly sought.

Respectfully submitted,

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A handwritten signature in black ink, appearing to read 'Lisa B. Kole', written over a horizontal line.

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